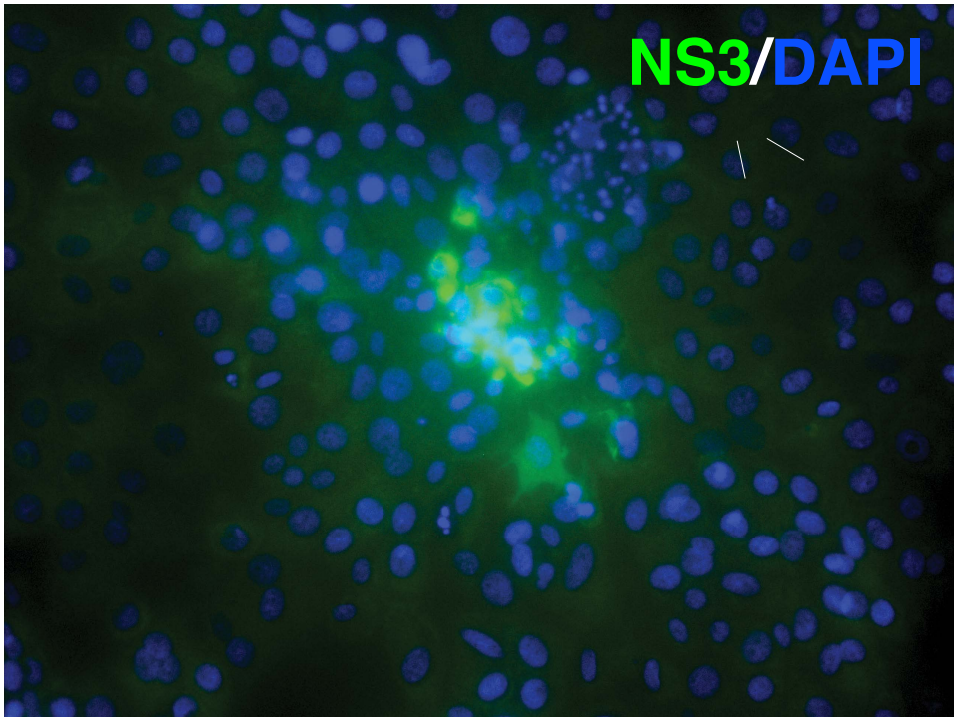
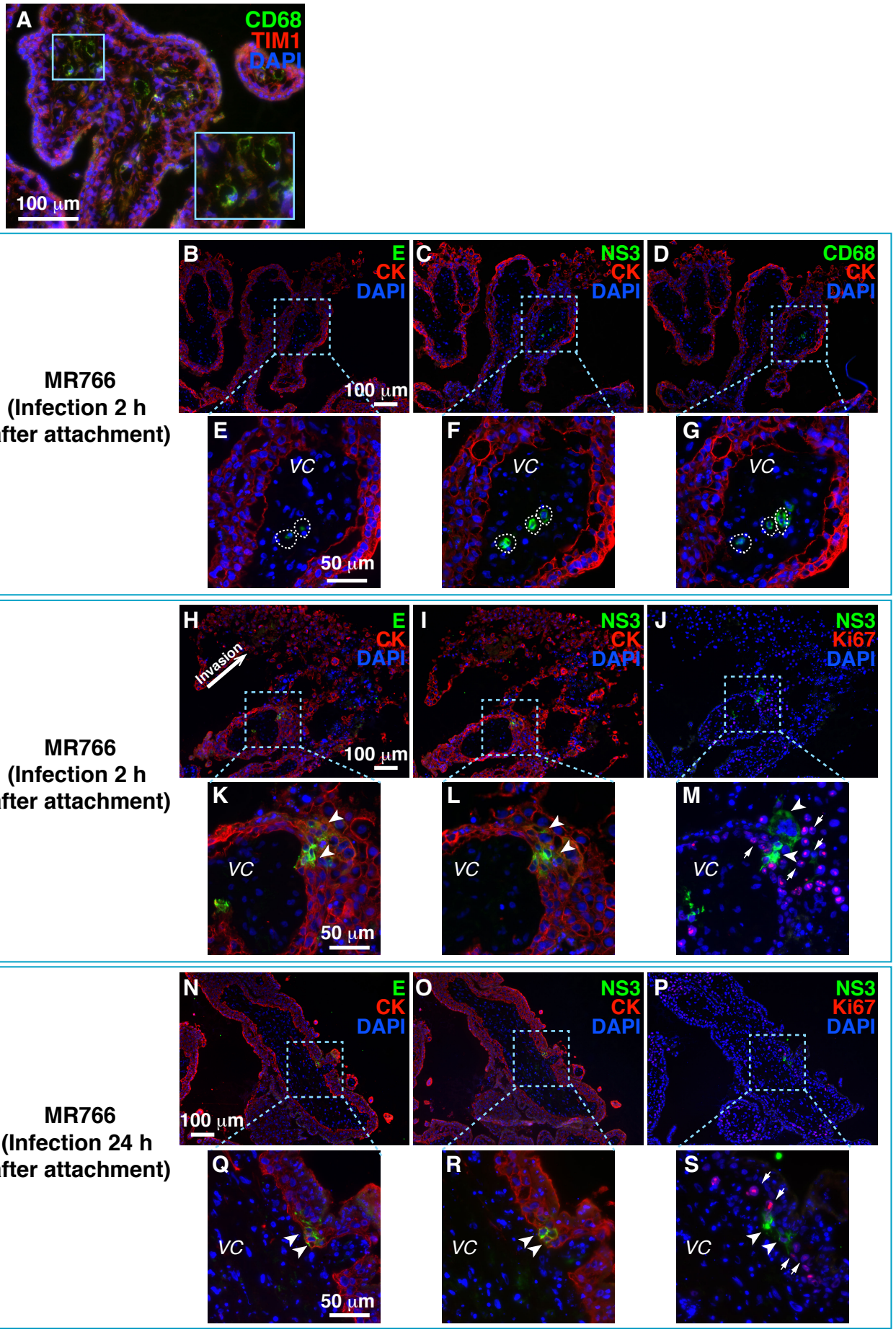
**Figure S1**



# Figure S2


A
CD68
TIM1
DAPI
100 μm
MR766
(Infection 2 h
after attachment)
B
E
CK
DAPI
100 μm
C
NS3
CK
DAPI
D
CD68
CK
DAPI
E
VC
50 μm
F
VC
G
VC
MR766
(Infection 2 h
after attachment)
H
E
CK
DAPI
Invasion
100 μm
I
NS3
CK
DAPI
J
NS3
Ki67
DAPI
K
VC
50 μm
L
VC
M
VC
MR766
(Infection 24 h
after attachment)
N
E
CK
DAPI
100 μm
O
NS3
CK
DAPI
P
NS3
Ki67
DAPI
Q
VC
50 μm
R
VC
S
VC

**Supplemental Figure Legends.**

**Figure S1. ZIKV MR766 infects primary differentiating CTB from midgestation placentas**. CTB were plated on Matrigel and immunostained for NS3 protein at 96 hpi. Nuclei in blue (DAPI). **Related to Figure 1.**

**Figure S2. ZIKV MR766 and Nica1-16 strains infect chorionic villus explants.** Chorionic villus explants were plated on Matrigel and either infected or mock-infected with MR766 at 2 or 24 h after attachment to the Matrigel then fixed and immunostained at 3 days after plating (i.e., 2 or 3 dpi). (A) Uninfected control explant (8.1 wk) immunostained for TIM1 (red) and CD68 (green), a marker of Hofbauer cells. TIIM1 was detected in CD68-positive cells. (B-G) A 7.5 wk explant infected with MR766 2 h after attachment and immunostained for cytokeratin (CK; MAb 7D3), ZIKV proteins E (B, E) and NS3 (C, F) and CD68 (D, G) in adjacent sections. Both E and NS3 were detected in CD68-positive cells, circumscribed by dotted lines. Chorionic villus explants (8.4 wk) were infected with MR766 2 h (H-M) or 24 h (N-S) after attachment to Matrigel, and adjacent sections were immunostained at 3 days after attachment for E protein and cytokeratin (CK; 7D3 MAb) (H, inset K, N, inset Q), NS3 and CK (I, inset L, O, inset R) and NS3 and Ki67 (J, inset M, P, inset S). In explants infected 2 h after attachment, ZIKV proteins were detected in proliferating CTB (K and L, arrowheads). In explants infected 24 h after attachment, ZIKV protein-positive cells were detected in underlying CTB (Q and R, arrowheads). NS3-positive cells (M and S, arrowheads) were in a zone of Ki67-positive proliferating cells (arrows). Nuclei in blue (DAPI). VC: villus core. **Related to Figure 1.**

**Table S1. Titers of prototype and Nicaragua ZIKV strains in different cell types of the human placenta. Related to Figure 1.**

| ZIKV strain | dpi [a] | AmEpC (gestation week)[b] | | | | TBPC[c] | HPF[d] | HUVEC[d] | CTB D206[d] | CTB D207[e] |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 21.2 | 22.6 | 38.6 | 40.2 | | | | | |
| **Uganda MR766** | **3** | $2x10^6$ | $4x10^6$ | $4.5x10^5$ | $4x10^5$ | $7.5x10^5$ | $3x10^6$ | $2.5X10^6$ | $2.7x10^3$ | $1.0x10^5$ |
| | **5** | $3.5x10^6$ | $2.7x10^7$ | | $3.5x10^5$ | $1.5x10^6$ | $2.7x10^6$ | $1.5x10^6$ | ND | ND |
| | **7** | $3.75x10^6$ | $2x10^6$ | | $5.2x10^4$ | $1.75x10^5$ | $3x10^6$ | $5x10^6$ | ND | ND |
| **Nica 1-16** | **3** | $1.00x10^5$ | $8.75x10^5$ | $3.75x10^4$ | $2x10^4$ | $1x10^4$ | | | | |
| | **5** | $1.75x10^5$ | $1.63x10^5$ | $3.38x10^4$ | $2.75x10^4$ | $1.5x10^4$ | ND | ND | ND | ND |
| | **7** | $2.0x10^5$ | $2.88x10^5$ | $4.35x10^4$ | $1.75x10^4$ | $1.75x10^5$ | | | | |

[a]dpi, days postinfection. [b]Infected at MOI 0.001; [c]Infected at MOI 1 (MR766) and MOI 0.004 (Nica1-16); [d]Infected at MOI 1; [e]Infected at MOI 2. Titers were determined by FFA (FFU/ml) on BHK cells after 3 days.

**Table S2. Summary of Axl, Tyro3 and TIM1 immunohistochemical staining in biopsies of human placenta and fetal membranes. Related to Figure 5.**

| Donor | GA (wk) | Axl | | | | Tyro3 | | | | TIM1 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | AmEpC | Ch | Villi | Decidua | AmEpC | Ch | Villi | Decidua | AmEpC | Ch | Villi | Decidua |
| AM5 | 22 | – | | | | ++ | | | | ++ | | | |
| AM6 | 20 | +++ | | | | + | | | | ++ | | | |
| AM7 | 22 | ++ | | | | + | | | | +++ | | | |
| AM8 | 23 | ++ | | | | + | | | | | | | |
| AM12 | 17 | +++ | | | | +++ | | | | +++ | | | |
| AM13 | 21 | +++ | | | | | | | | | | | |
| AM18 | 27 | +++ | | | | + | | | | +++ | | | |
| AM19 | 18 | +++ | | | | + | | | | +++ | | | |
| SF147 | 23 | ++ | + | + | ++ | | | | | | | | |
| SF148 | 17 | ++ | + | + | ++ | | | | | | | ++ | ++ |
| SF149 | 23 | ++ | + | + | ++ | | | | | | | ++ | ++ |
| UCSF7 | 19 | | | + | ++ | | | | | | | | |
| AM17 | 40 | — | | | | ++ | | | | ++ | | | |
| SF34 | 39 | — | ++ | + | +++ | | | | | | | | |
| SF38 | 39 | — | ++ | + | +++ | | | | | ++ | ++ | ++ core STB | ++ CTB |
| SF66 | 32 | — | ++ | + | +++ | | | | | | | | |
| SF70 | 32 | — | ++ | + | +++ | | | | | | | | |
| SF88 | 39 | | | | | | | | ++ CTB | ++ | ++ | ++ core STB | ++ CTB |
| SF92 | 41 | — | ++ | ++ | | | | | | | | | |
| SF95A | 36 | — | ++ | + | | | | | | | | | |
| SF102 | 39 | — | ++ | + | | | | | | | | | |
| SF105 | 32 | — | | | | | | | | ++ | + | ++ core STB | +++ CTB |
| SF107 | 36 | — | | | ++ | ++ | ++ | — | +++ CTB | ++ | + | ++ core STB | ++ CTB |
| SF111 | 40 | — | | | ++ | +++ | | | +++ CTB | +++ | | | +++ CTB |
| SF114 | 37 | — | ++ | + | +++ | ++ | | | +++ CTB | | | | |
| SF126A | 37 | — | | | +++ | ++ | +++ | — | +++ CTB | ++ | + | ++ core STB | ++ CTB |
| SF131 | 36 | | | | | | | | | +++ | | | ++ CTB |
| SF132 | 36 | | | | | | | | | +++ | | | ++ CTB |
| SF133 | 38 | | | | | | | | | +++ | | | ++ CTB |
| SF107 | 36 | | | | | ++ | ++ | — | +++ CTB | + | + | ++ core STB | ++ CTB |

STB, sycytiotrophoblasts. CTB, cytotrophoblasts. Ch, chorion. The donor number is shown in the first column.

**Table S3. Summary of cofactor expression by tissue/cell type. Related to Figures 3 and 5.**

| | AmEpC | Chorion | Villi | Decidua | CTBs |
|---|---|---|---|---|---|
| **Biopsies** | | | | | |
| **AXL** | 10/24 | 11/11 | 12/12 | 12/12 | 3/3 |
| **TYRO3** | 13/13 | 3/3 | 0/3 | 6/6 | 6/6 |
| **TIM1** | 17/17 | 6/6 | 8/8 | 12/12 | 10/10 |
| **Primary cells** | | | | | |
| **AXL** | 11/13 | NA | NA | NA | 4/7 |
| **TYRO3** | 9/13 | NA | NA | NA | 7/7 |
| **TIM1** | 13/13 | NA | NA | NA | 7/7 |

## Supplemental Experimental Procedures

### Antibodies and reagents

Commercial antibodies used were: goat polyclonal anti-Axl and -GATA4 (R&D Systems); rabbit polyclonal anti-Tyro3, -TIM1, -vimentin, and -Ki67 (Abcam), anti-von Willebrand factor (Thermo Scientific), and anti-cytokeratin 19 (Proteintech); and mouse anti-TIM1 (Abcam), anti-CD209 (BD Pharmingen), anti-CD68 and -cytokeratin 7 (Dako), anti-CD31 (Fitzgerald) and anti-actin (Sigma-Aldrich). Rat anti-human cytokeratin MAb (clone 7D3) and mouse anti-MHC class I HLA-G MAb (clone 4H84) were gifts from Susan Fisher (UCSF) (Damsky et al., 1992) and Michael McMaster (UCSF).

### Immunofluorescence staining

Cells grown on cover slips were fixed with 4% PFA and permeabilized with 0.1% Triton X-100. Frozen tissues (5 cases) were cut into 5-μm sections. After blocking with bovine serum albumin (BSA) or 3-5% normal serum matching the secondary antibody source, primary antibodies were incubated overnight followed by secondary antibodies (fluorescein isothiocyanate or rhodamine red-X conjugates; Jackson ImmunoResearch). Images were obtained using a Nikon Eclipse 50i microscope with a SPOT 7.4 Slider camera (Diagnostic Instruments). Images were processed and analyzed using Adobe Photoshop and NIH Image J software.

### Immunoblotting

Whole cell lysates were obtained using RIPA buffer (50 mM Tris [pH 7.4], 150 mM NaCl, 1% [v/v] Nonidet-P40, 1 mM EDTA, 0.1% [w/v] SDS, and 1% Na-deoxycholate) supplemented with complete protease inhibitor cocktail (Roche), and immunoblot analysis was performed as described previously (Tabata et al., 2015).

### Statistical analysis

An unpaired t-test was used for pair-wise statistical comparisons and a one-way ANOVA with Dunnett's post-test for multiple comparisons. The mean +/- standard deviation (SD) is shown in all bar- and dot plots. All statistical analyses were performed in GraphPad Prism v6.0.